

DEVELOPMENT OF A TECHNIQUE FOR METABOLIC
INVESTIGATIONS AND ITS APPLICATION
TO STUDIES WITH RHODOSPIRILLUM RUBRUM

ELSIE O. ELLINGSON

Library
U. S. Naval Postgraduate School
Monterey, California

1-55

MET METABOLISM

RHO RHODOSPIRILLUM RUBRUM

Perry G. Davis BOOKBINDING

2216 CLEMENT STREET San Francisco 21, Calif. BA. 1-6352

DEVELOPMENT

DIRECTIONS FOR BINDING

ITS APPLIC

BUCKRAM

COLOR NO. 8854

FABRIKOID

COLOR

LEATHER

COLOR

Letter in Gold

OTHER INSTRUCTIONS

Letter on front cover:

DEVELOPMENT OF A TECHNIQUE FOR METABOLIC
INVESTIGATIONS AND ITS APPLICATION
TO STUDIES WITH RHODOSPIRILLUM RUBRUM

ELSIE O. ELLINGSON

LETTERING ON BACK
TO BE EXACTLY AS
PRINTED HERE.

ELLINGSON

1955

THESIS
E35

DEVELOPMENT OF A TECHNIQUE FOR METABOLIC INVESTIGATIONS
AND
ITS APPLICATION TO STUDIES WITH RHODOSPIRILLUM RUBRUM

* * * * *

Elsie O. Ellingson

and

Harold E. Shaw

DEVELOPMENT OF A TECHNIQUE FOR METABOLIC INVESTIGATIONS
AND ITS APPLICATION TO STUDIES WITH RHODOSPIRILLUM RUBRUM

by

Elsie Ovedia Ellingson
Major, United States Air Force

and

Harold Edwin Shaw
Captain, United States Army

Submitted in partial fulfillment
of the requirements
for the degree of

MASTER OF SCIENCE
IN
PHYSICS

United States Naval Postgraduate School
Monterey, California

1 9 5 5

Thesis

E 35

This work is accepted as fulfilling
the thesis requirements for the degree of

MASTER OF SCIENCE

IN

PHYSICS

from the

United States Naval Postgraduate School

PREFACE

The investigations reported herein were done in the physics laboratory of the U.S. Naval Postgraduate School, Monterey, California in the spring of 1955 for the purpose of assisting in the research studies of Dr. Roderick K. Clayton.

The writers wish to express their sincere appreciation to their advisor, Dr. Clayton, for his invaluable aid during this project. It was because of his suggestions, encouragement and editorial assistance that this work was completed.

Appreciation is due Dr. Ernest Dobson of the University of California for consenting to be the "second reader" of this paper, to Mr. Milton K. Andrews, OMC Robert C. Moeller, Mr. John Chitwood and Mr. Thomas Burson for their aid in obtaining necessary equipment, and to Mrs. Evelyn New for her assistance in preparing the manuscript.

This work was supported in part by the Office of Naval Research and the Armed Forces Special Weapons Project.

TABLE OF CONTENTS

Item	Title	Page
Chapter I	Introduction	1
Chapter II	Apparatus	4
Chapter III	Pyruvate Metabolism	10
Chapter IV	Propionate Metabolism	15
Bibliography		21
Appendix I	Culture and Preparation of the Bacteria. . . .	22
Appendix II	Pyruvate Assay	23

LIST OF ILLUSTRATIONS

Figure		Page
1	Open System	5
2	Closed System	7
3	Pyruvate Metabolism, Open System.	12
4	Pyruvate Metabolism, Closed System.	14
5	Correction Factors for Adding NaHCO_3	16
6	Examples of Propionate Metabolism.	18
7	Examples of Propionate Metabolism	19

CHAPTER I

INTRODUCTION

For many years investigators have been prying into the secrets of the photosynthetic bacteria. The similarities of these bacteria to members of both the plant and animal kingdoms make them an ideal subject for research into fundamental mechanisms of growth and behavior.

Rhodospirillum rubrum, which was investigated in this study, is one of these photosynthetic species. It is a spiral-shaped non-sulphur purple bacterium belonging to the family *Athiorhodaceae* according to the classification of Molisch [7]. Extensive investigations have been made by van Niel [9] and others on the isolation, nutrition, and photosynthetic metabolism, and by Manten [6], Clayton and Delbruck [2], and Clayton [1] on phototactic responses to *Rh. rubrum*.

One of the interesting facets of the metabolism of *Rh. rubrum* is that the organism is capable of utilizing many different simple substrates, and that the color, appearance and size of the organism may vary when grown in different media. Van Niel [9] has shown that the organism can be grown in the presence of most of the lower fatty acids and several amino acids, but that carbohydrates and their corresponding polyalcohols are utilized poorly or not at all. Further investigations have shown that many of the metabolic processes involve the Krebs tricarboxylic acid cycle. This was indicated by Eisenberg [3] when he stated,

The presence of all enzymes required for the oxidation of the Krebs cycle members, and the ability of the dried cells to form citric acid from acetic and pyruvic acids, indicate that the tri-carboxylic acid cycle is probably the pathway for the terminal oxidation of these acids.

Investigations using radioactive isotopes of carbon have indicated that, at least in some cases [5], oxidative metabolic processes in the dark are similar to those carried out under anaerobic conditions in the light. The work undertaken in this study was done to extend the information available on the basic processes involved in oxidative metabolism, utilizing two substrates: sodium pyruvate and sodium propionate.

Sodium pyruvate has been used before as a substrate in several studies on photosynthetic metabolism. It was hoped that information could be obtained on the inhibitory action of methylene blue by isolating and identifying the by-products formed during oxidative metabolism in the presence of this drug. However, as the experiments proceeded, it appeared that there were no organic by-products formed under these conditions, the only apparent effect of the methylene blue being a reduction in the rate of metabolism. The bacteria were tested for the presence of catalase, to check the possibility that H_2O_2 formed by the addition of methylene blue was the cause of the inhibition. Although catalase was found to be present, it is possible that the H_2O_2 is formed at too rapid a rate to be decomposed enzymatically.

Although sodium propionate is a favorable substrate for photosynthetic metabolism, the route by which it enters the Krebs cycle has not previously been reported for *Rh. rubrum* and little is known about its oxidative metabolism. An outstanding effect of using sodium propionate as a substrate for photosynthesis was demonstrated by Clayton [1]. The saturating light intensity for phototactic responses of *Rh. rubrum* is about four times as great in the presence of sodium propionate as in the presence of other substrates. Manten [6] and Clayton [1] have shown that the phototactic responses of *Rh. rubrum* depend on changes in the rate of

photosynthetic metabolism. Therefore it was believed that a study of the metabolism of propionate might lead to a better understanding of the reason for high saturating light intensity with propionate, and of the manner in which tactic responses are mediated chemically.

An investigation of oxidative metabolism utilizing sodium propionate showed that there was no appreciable utilization aerobically and in the dark, unless CO_2 was introduced in some manner, although the amount of CO_2 necessary was extremely small. It was also found that, after an initial addition of CO_2 , the metabolism became self-sustaining at a high rate even under CO_2 -free conditions. This indicates that the probable mode of metabolism of sodium propionate is by the carboxylation of propionic acid to succinic acid, entering the Krebs cycle as such. Once the reaction has begun, the necessary CO_2 is provided by successive oxidations occurring within the cycle.

For the investigation of these metabolic processes, apparatus was developed which would permit continuous control of the gaseous phase of the system and would facilitate withdrawal of samples for analysis. The essential feature was a light-tight container for the bacterial suspension, through which a gas of controlled composition could be bubbled. The metabolism involved the liberation of alkali, which could be followed by means of a pH meter. In addition, the apparatus permitted a collection and analysis of the effluent gases.

In many respects the apparatus, described in detail in Chapter II, has advantages over the familiar Warburg-Barcroft technique, in that it provides for simpler procedures, continuing analyses during an experiment, and control of the gas phase.

CHAPTER II

APPARATUS

For the experiments performed during these investigations on oxidative metabolism, two different systems were employed. For simplicity, these will be designated as the open and the closed systems.

The open system was so designed that up to four experiments or runs could be made at one time if desired. One unit of this system is shown in cross section in Figure 1. A box painted black on the inside was used to hold a 250 ml. beaker containing the bacterial suspension¹. Three holes in the lid permitted entrance of a glass dispersion tube for bubbling air through the suspension, a buret for the addition of acid, and the electrodes of a pH meter. Light baffles were placed around the entrances for the dispersion tube and the buret. The underside of the lid and the top of the box were covered with black velvet to prevent entrance of light.

A Beckman pH meter was used and was mounted on a sliding platform to facilitate moving the electrodes from one box to another in case of multiple runs. A separate lid lined with black velvet was made to cover the "electrode opening" during intervals when the electrodes were not in position. While the electrodes were in position in a suspension, a black focussing cloth was draped around them to prevent any light from entering the box.

At an early point in the experimental work it became evident that it would be desirable to control the composition of the air above the medium and to devise some means of measuring the output of carbon dioxide from

1. See Appendix I.

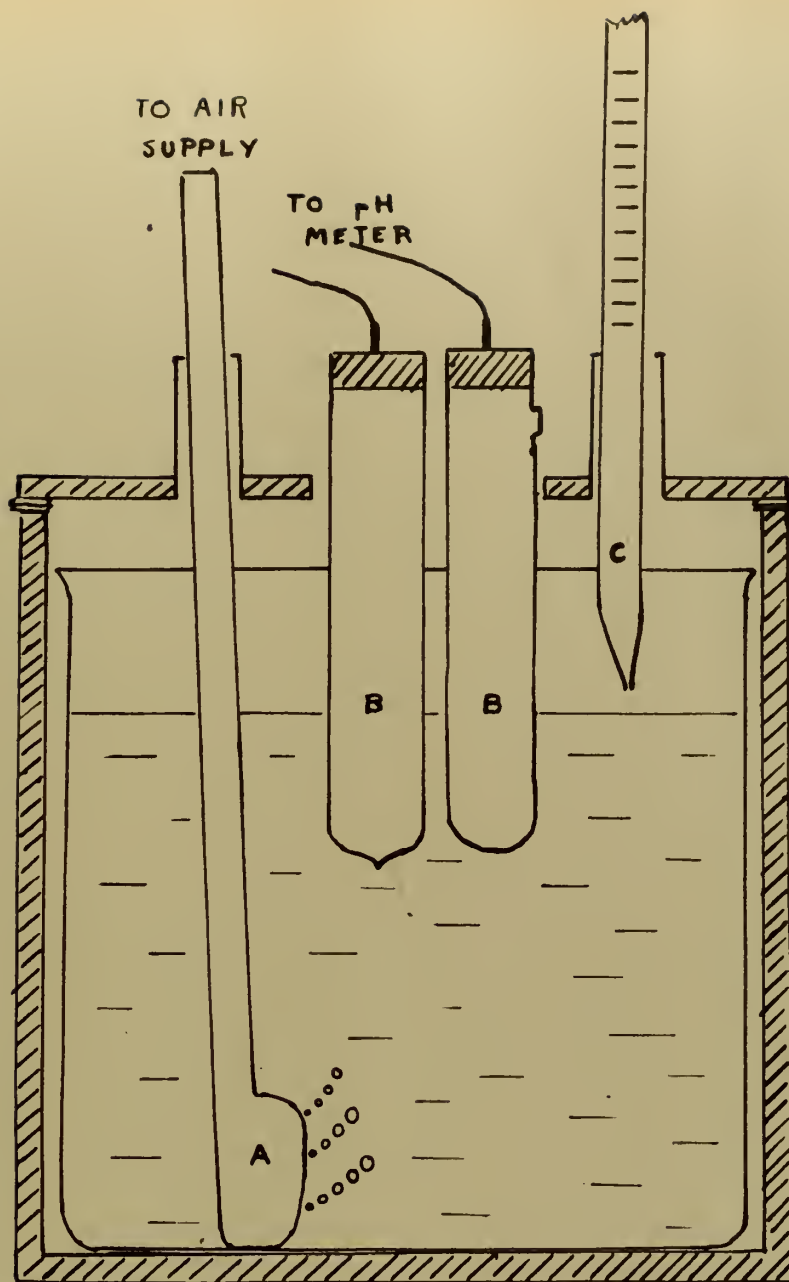


Figure 1. Open System

A-Sintered glass dispersion tube, B-electrodes of pH meter, C-buret.

the system during the metabolism of the substrate under investigation. To accomplish these objectives the closed system shown schematically in Figure 2 was constructed. It was also possible with this system, to determine qualitatively if any acetaldehyde was produced during the metabolism by bubbling the effluent gases through a solution of 1/5-saturated 2,4-dinitrophenylhydrazine in 2 N HCl. As little as 1 μ mol of acetaldehyde will show a yellow precipitate.

The system was built around a paraffin-coated aluminum cylinder 6 cm. in diameter and 12 cm. in length (F) fitted at each end with a rubber stopper. It was in this light-proof vessel that the metabolism of *Rh. rubrum* was carried out. The electrodes of the pH meter (H) were inserted through holes bored in the stopper at the top of the cylinder. In this way the pH of the suspension could be determined at all times. A buret (E) was inserted through the upper stopper to enable the operator to control the pH of the suspension. It was necessary to connect the top of the buret with the trap (L) to equalize the pressure above and below the acid in the buret. A length of glass tubing fitted with a short piece of rubber tubing (I) was also inserted through the upper stopper to allow addition of the substrate, after flushing the system with CO₂ free-air, without opening the entire chamber to the atmosphere. This addition was accomplished by fitting a pipette into the rubber tubing while reducing the pressure in the system, thus permitting the substrate to run down into the chamber. The rubber tubing was clamped tightly at all other times. A final hole bored in the upper stopper was fitted with a small, inverted test tube (G). This permitted the air outlet tube to extend well above the level of liquid in the chamber to prevent spilling over into the trap.

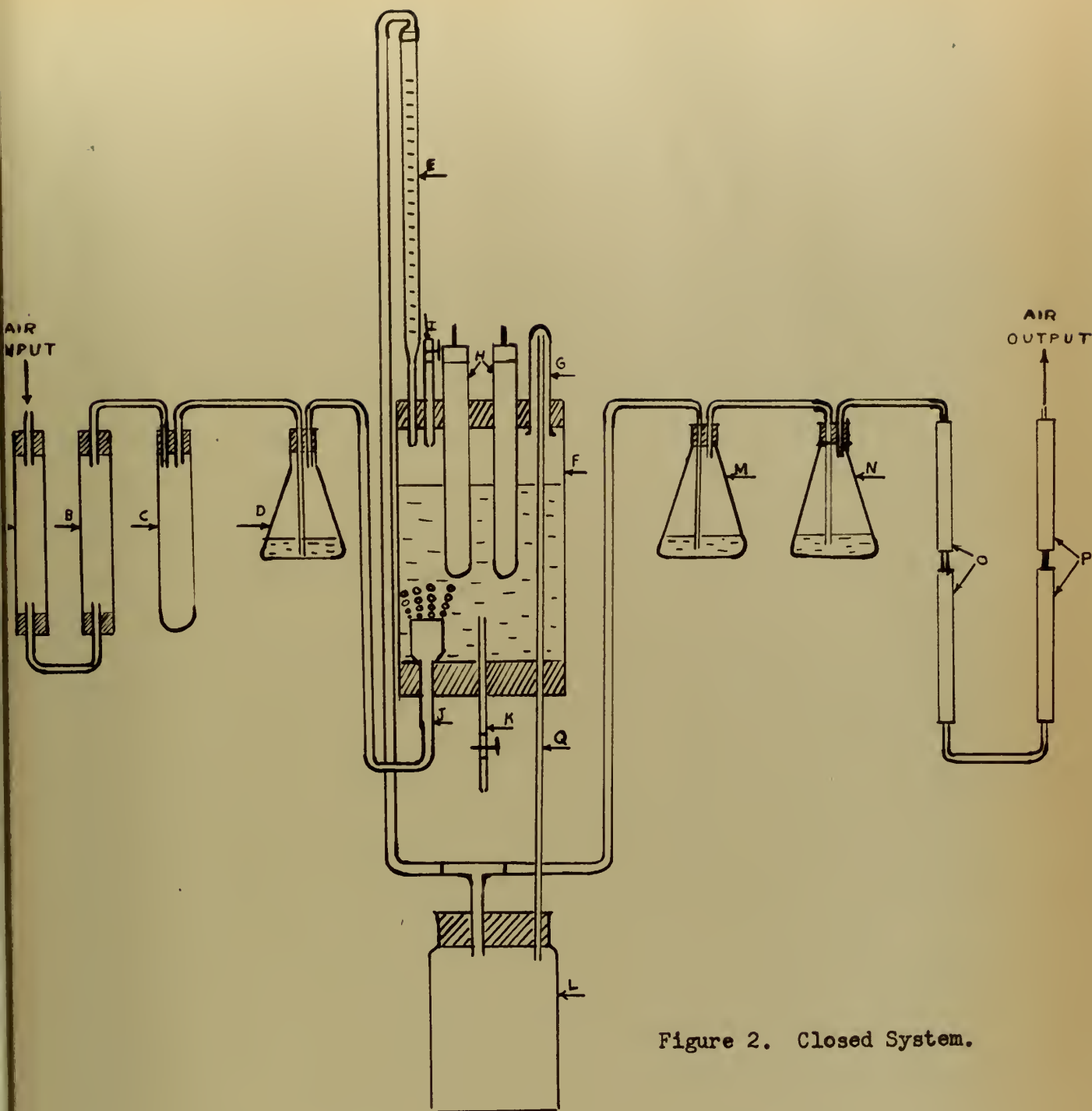


Figure 2. Closed System.

The lower stopper was fitted with a gas dispersion tube (J) to bubble the input air throughout the suspension, flushing it thoroughly and continuously. In addition, this stopper was fitted with a small drain (K) permitting withdrawal of a sample at any time, and an air outlet tube (Q) connecting the chamber with the trap. This prevented a build-up of pressure in the cylinder.

The air input was channeled through a tube of dessicant (A) and then through another tube filled with Ascarite (B) to remove the CO_2 . The dessicant tube was provided to prevent the absorption of water vapor by the Ascarite with subsequent caking. The air was then "moistened" again by bubbling it through 0.1 N H_2SO_4 (D). This served to resaturate the air, preventing a reduction in volume of the suspension during the run. The air was then fed into the chamber through the dispersion tube (J).

The effluent air was passed through a bath of concentrated H_2SO_4 (N) which removed most of the water vapor from the gas. That moisture which was not removed was taken up in anhydrous CaCl_2 (O). The resulting dry gas was then passed through Ascarite (P) to determine the CO_2 content. Provision was made in the design to permit addition of a flask (M) containing 1/5 saturated 2,4 dinitrophenylhydrazine in 2 N HCl to determine qualitatively the presence of acetaldehyde in the effluent gas.

The dessicant and Ascarite tubes (O & P) were removed and weighed before and after the experiment to determine the CO_2 production during metabolism. It was found that the second dessicant tube showed no increase in weight indicating that all moisture had been removed and that the increase in weight of the Ascarite was attributable solely to the CO_2 .

The original design provided for the buret to feed into the chamber

through the lower stopper. This plan was modified to prevent diffusion between the acid in the buret and the suspension. It was also found necessary to connect the pressure vent on the side of the calomel electrode to the trap with a length of rubber tubing. Without this provision the elevated pressure in the chamber forced the suspension into the salt bridge of the electrode resulting in erratic behavior of the pH meter.

The ability to maintain a constant and controllable gas phase, and the relative ease of withdrawal of samples during a run, result in a considerable advantage over the Warburg-Barcroft manometric technique previously used for this type of investigation. The apparatus lends itself admirably to the use of larger volumes of suspension, facilitates supplemental analyses during the run, and results in a simpler experimental procedure. Of practical advantage is the relative ease of assembly and of cleaning.

A possible application of the system, which was not attempted in this investigation, would be in the field of radioisotopes. If a means of collecting a volatile isotope in a form suitable for counting, such as a disc of Ascarite, were devised, the usefulness of the system could be greatly extended. This would permit the collection of C^{14} labeled CO_2 .

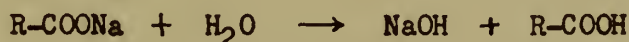
The ease of addition of alkali or acid to the bacterial suspension suggests that other investigations, dependent on volumetric techniques, could be devised for this apparatus. The possibility of analysis of effluent vapors containing various volatile organic compounds is also indicated. This could easily be accomplished by the use of a combustion train connected to the air outlet.

CHAPTER III

PYRUVATE METABOLISM

Originally the studies of oxidative metabolism of sodium pyruvate by *Rh. rubrum* were undertaken to determine whether organic by-products were formed in the presence of methylene blue. However, results of experiments performed during the same period by Clayton (verbal communication) indicated that no by-products were formed and that the sodium pyruvate was utilized completely by the bacteria. The latter was also indicated by the experiments described below.

Two similar procedures were used throughout these studies on oxidative metabolism. Air was bubbled through a suspension of bacteria¹ which was placed in a light-tight box (open system) or an aluminum cylinder (closed system). The metabolism was studied by following the production of alkali by the bacteria; during the metabolism reactions occur which are generalized below, with the final organic product being assimilated or oxidized:



The suspension was kept between a pH of 6.8 and 7.3. One tenth normal H_2SO_4 was added in sufficient quantities to bring the pH slightly below 7.0. The time and the amount of acid added were recorded when the pH of the suspension again reached a value of 7.0. At various intervals coincident with a pH reading, samples of the suspension were withdrawn for a pyruvate assay². Results of the pyruvate assays and the amounts of alkali produced were plotted against the elapsed time from the beginning of a run.

1. See Appendix I.

2. See Appendix II.

Figure 3 is an example of a typical study in which two runs were made simultaneously using the open system, with bacteria from the same culture. As can be seen from the figure, a change in the rate of metabolism was obtained when the suspension was poisoned with 2 cc. of 1% methylene blue. However, the alkali production followed the utilization of sodium pyruvate in both cases, indicating that no other organic sodium salt was formed during the metabolism. Also, almost complete utilization of the substrate occurred since 500 μ moles of sodium pyruvate were introduced in each case.

A possible explanation for the suppression of metabolic rate in the presence of methylene blue was considered to be the production of H_2O_2 . If the bacteria did not have the enzyme, catalase, which decomposes H_2O_2 in many cells, the H_2O_2 formed from the methylene blue could be toxic to the bacteria, and thus decrease the rate of metabolism. However, a mass of centrifuged bacteria were tested with H_2O_2 and the presence of catalase in the bacteria confirmed. Another possibility is that the production of H_2O_2 is too rapid for enzymatic actions to neutralize its effect. This could be investigated by adding catalase to the suspensions; this was not undertaken in the present studies.

Two runs were made using the closed system for the purpose of determining the amount of CO_2 produced during the metabolism. The procedure followed was essentially the same as that used in the open system and results were plotted in a similar fashion. However, all runs utilizing the closed system were plotted on a basis of μ mol/100 ml. suspension to keep the scale the same as those for the open system. Increase in the weight of the Ascarite tubes was 57 mg. in one run and 55 mg. in the other. Since

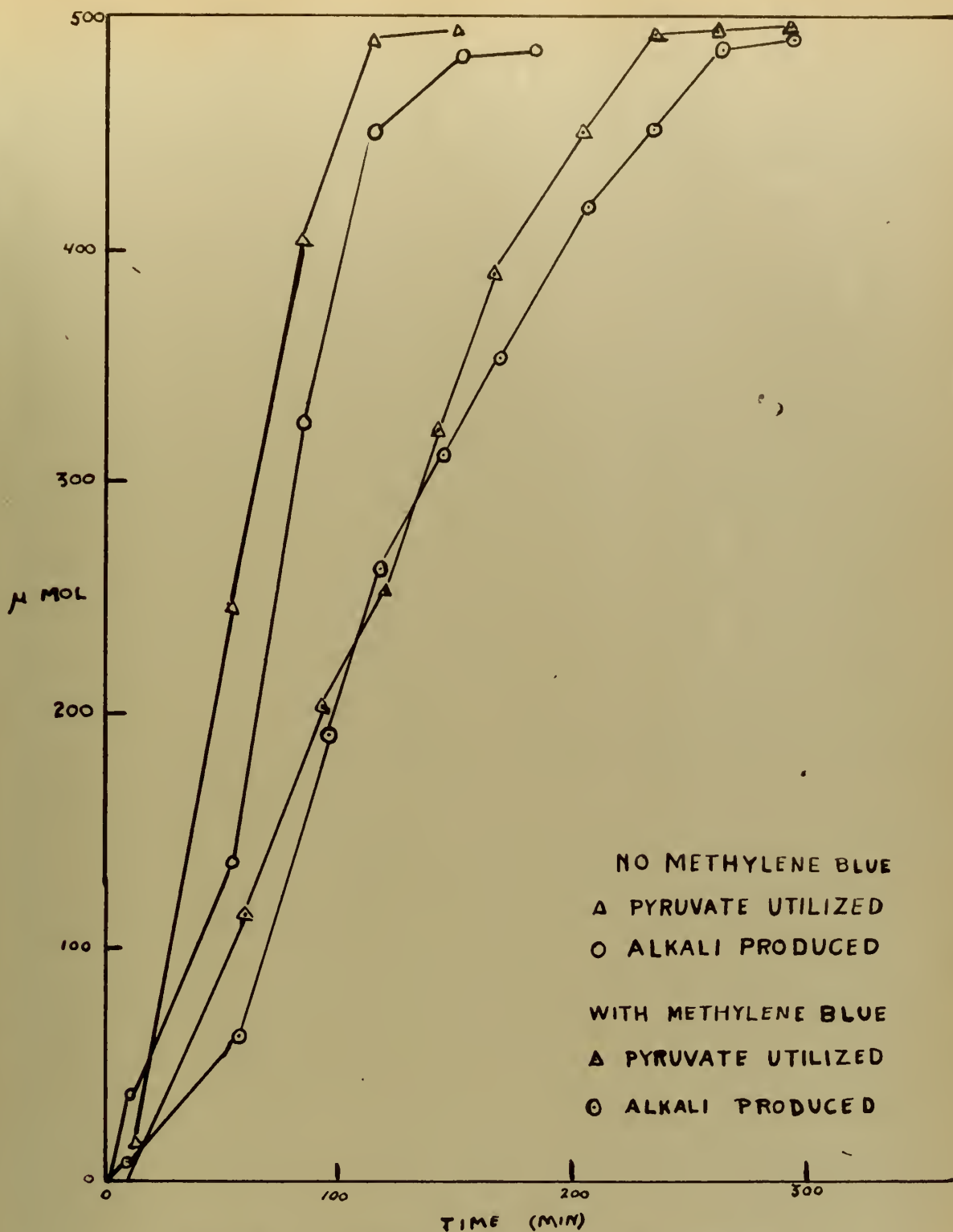


Figure 3. Pyruvate Metabolism, Open System.

750 μ moles of sodium pyruvate were used in each case, this would indicate that slightly less than half of the carbon in the substrate was assimilated and the balance oxidized to CO_2 , as shown below:

$$\text{Carbon added} = 750 \mu\text{moles} \times \frac{12 \mu\text{g}}{\mu\text{mol}} \times 3 \text{ Catoms} = 27000 \mu\text{g}^{\text{C}} \text{ or } 27 \text{ mg}^{\text{C}}$$

$$\text{Carbon oxidized to } \text{CO}_2 = 55 \text{ mg} \times \frac{12}{44} = 15 \text{ mg}^{\text{C}}$$

$$\% \text{ C oxidized} = \frac{15}{27} \times 100 = 55.6\%$$

Results of one of these runs are shown in Figure 4.

In both runs with the closed system the alkali production did not follow the disappearance of the sodium pyruvate to completion. At the present time, this discrepancy cannot be accounted for since all other evidence, including work done by Clayton using the Warburg technique, shows that the sodium pyruvate is completely assimilated or oxidized to CO_2 and H_2O . It is thought that nitrogen metabolism might be responsible in some manner for some acid production, but investigations along this line were not pursued.

In summary, the studies on oxidative metabolism of *Rh. rubrum* using sodium pyruvate as a substrate furnished the following information:

(1) Addition of methylene blue suppresses the rate of metabolism; (2) The poisoning with methylene blue does not lead to the accumulation of any organic end-products arising from sodium pyruvate; (3) Catalase is present in *Rh. rubrum*, but may not be present in sufficient quantities to neutralize the toxic effects of H_2O_2 produced by the methylene blue; (4) Production of CO_2 during the metabolism of sodium pyruvate indicates that approximately half of the carbon in the substrate is assimilated by the cell and half is oxidized.

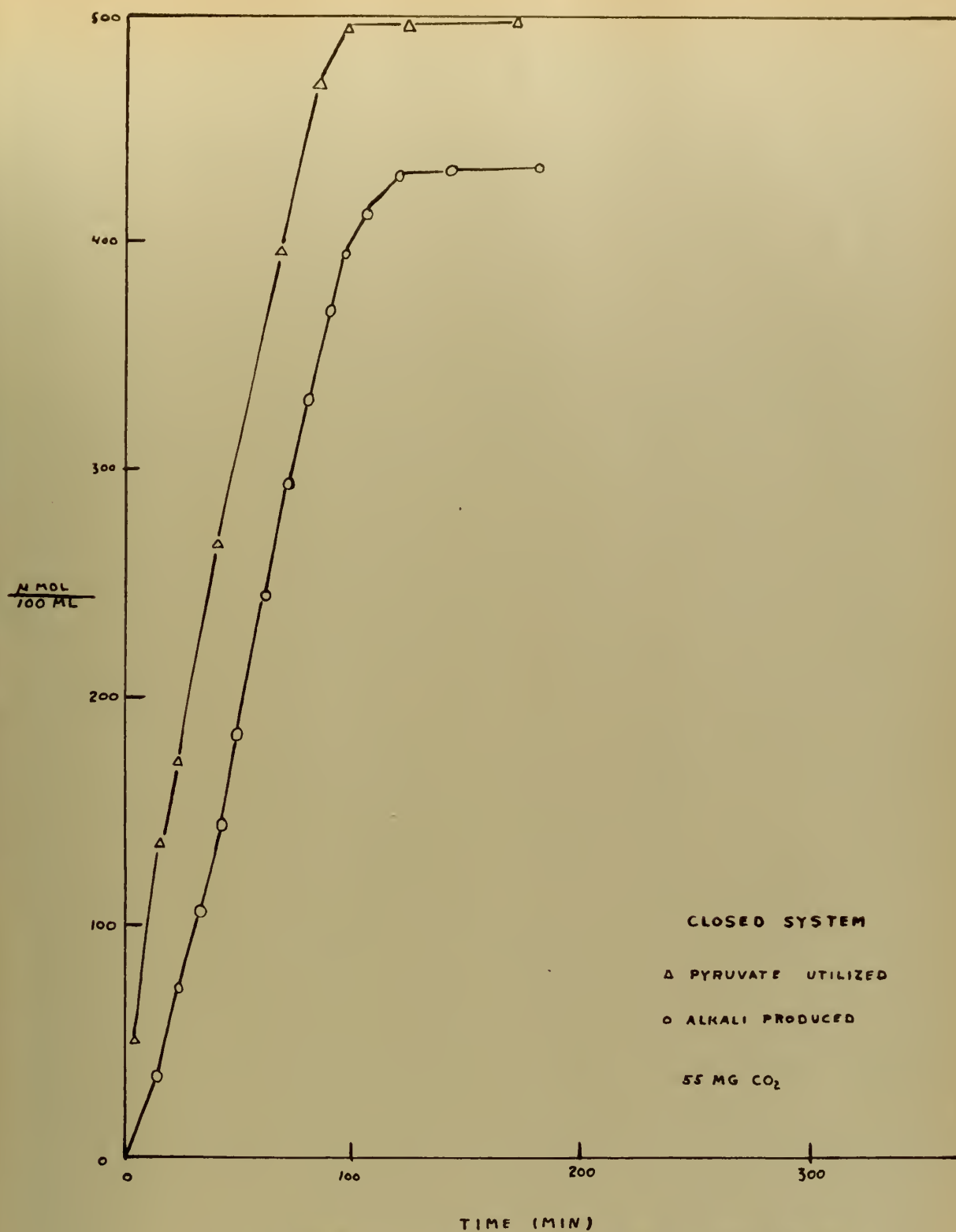


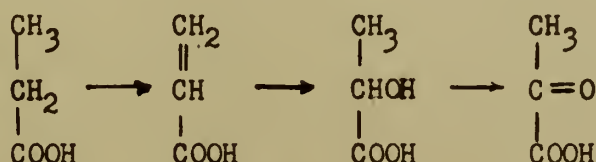
Figure 4. Pyruvate Metabolism, Closed System.

CHAPTER IV

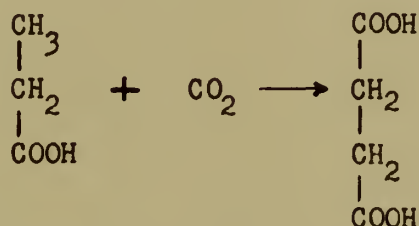
PROPIONATE METABOLISM

Little is known of the processes involved in the metabolism of propionate. If the theory of Eisenberg [3] is correct in that terminal oxidations are by means of the Krebs cycle, some mechanism must be involved for introducing propionate into the cycle.

Two reasonable hypotheses can be proposed to explain this reaction. On the one hand, propionic acid could enter the Krebs cycle by dehydrogenation, hydration and oxidation to pyruvic acid as shown schematically:



Alternatively, propionic acid could be carboxylated directly to yield succinic acid:



Since it had already been shown by van Niel [10] that metabolism of acetate in the dark by purple bacteria requires the presence of CO_2 , it was decided to explore the second possibility and determine whether CO_2 was required for the oxidative metabolism of sodium propionate.

The procedures used in making the studies with sodium propionate as a substrate were similar to those utilizing sodium pyruvate, with small variations. At times the substrate was added at the beginning of the run; in others the substrate was added only after the suspension had been

thoroughly flushed with CO_2 -free air. In several of the experiments, CO_2 was added during the run either in the form of sodium bicarbonate or by using air mixed with CO_2 . Air containing known percentages of CO_2 was prepared in a mixing bottle. As in the studies with sodium pyruvate, the utilization of the substrate was followed by observing the liberation of alkali. No samples of the bacterial suspension were withdrawn during the runs, as a suitable test for propionate had not been devised.

To determine how much of the alkali was formed from metabolism and how much was introduced as added sodium bicarbonate, a correction factor was determined by Clayton. This was accomplished by using a solution of the same concentration as the suspension but without the bacteria. Fifty μmol of sodium bicarbonate were added to 100 ml. of solution and a constant pH of 7.0 was maintained with 0.1 N H_2SO_4 . Correction factors in μmoles of alkali produced per 100 ml. of suspension were plotted for runs using normal air and CO_2 -free air, as shown in Figure 5.

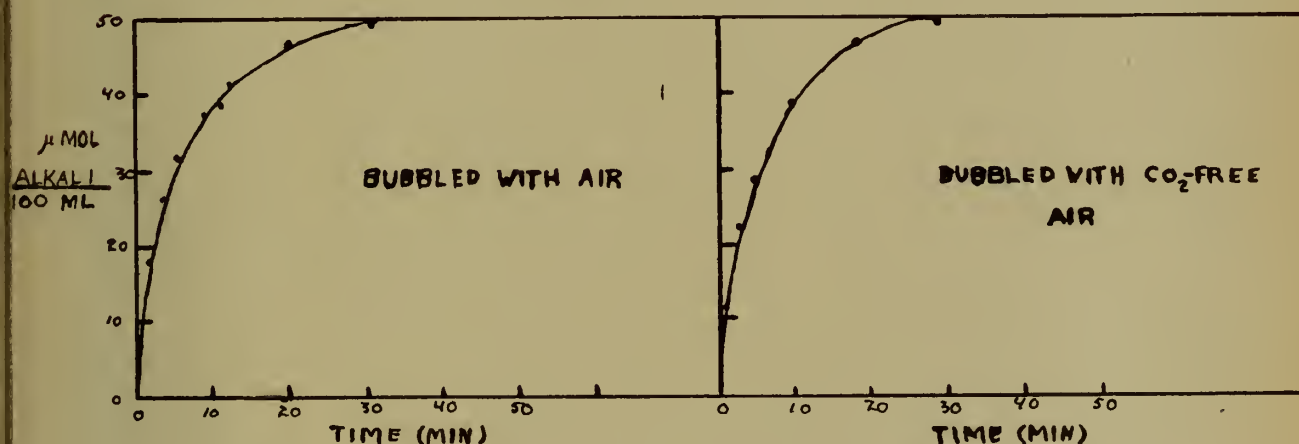


Figure 5. Correction factors for adding NaHCO_3 (50 μmol in 100 ml).

One of the first experiments utilized the closed system to permit only CO_2 -free air to pass through the bacterial suspension. For a period

of about two hours, no alkali was produced except that which could be accounted for by endogenous metabolism. After this time, 75 μmol s of NaHCO_3 were added and the run continued for about three hours. Results of this run are shown in Figure 6(a); crosses indicate experimental points corrected as outlined in the foregoing paragraph.

Several runs similar to the one described indicated that some CO_2 was necessary for the oxidative assimilation of sodium propionate. However, in others utilizing the open system and either normal air or CO_2 -free air, the rate of metabolism was nearly maximal at the beginning of the run, as can be seen in Figures 6(c) and (d). In one run using CO_2 -free air in the open system, the metabolic rate increased after about an hour from the time the substrate was added without the addition of any CO_2 [Figure 7(a)]. From the observations made, it could be assumed that only trace amounts of CO_2 are necessary for the metabolism of propionate. In the case where the rate eventually increased without addition of any CO_2 , it is possible that endogenous metabolism might be providing the necessary CO_2 . Rates of air flow through the suspension might also be a factor in the accumulation of CO_2 , as this flow was not rigidly controlled. In any event, the addition of CO_2 always produced an increase in rate, when this was not maximal beforehand.

During several experiments, however, after metabolism had reached a constant rate with air containing some CO_2 , air free from CO_2 was used for the remainder of the run or the concentration of CO_2 in the air was changed [Figures 7(b) and 6(b) and (d)]. In these latter cases, no change in the rate of metabolism was detected after the maximum rate was once established. Therefore, it seems quite definite that the reactions are self-sustaining

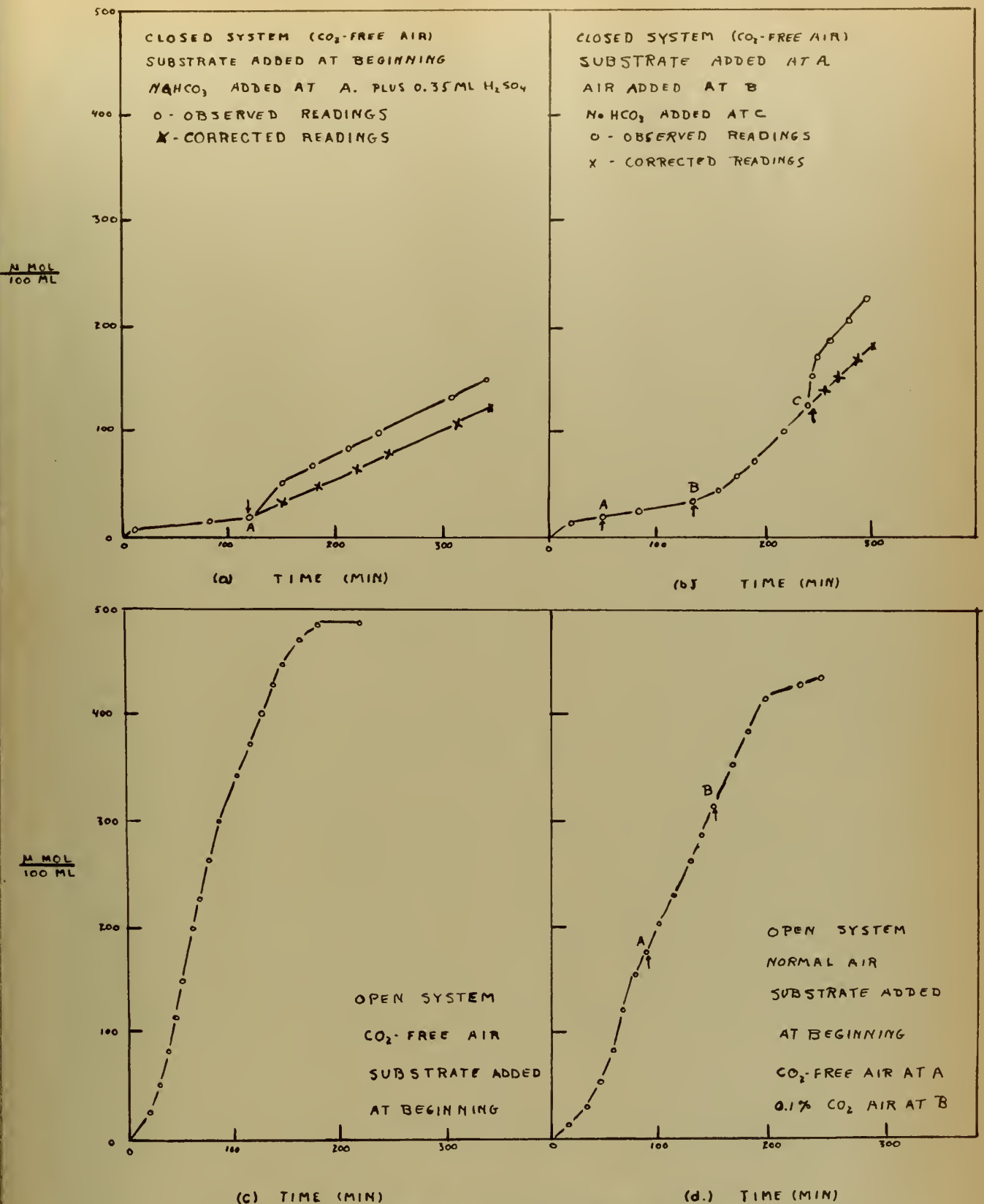


Figure 6. Examples of Propionate Metabolism

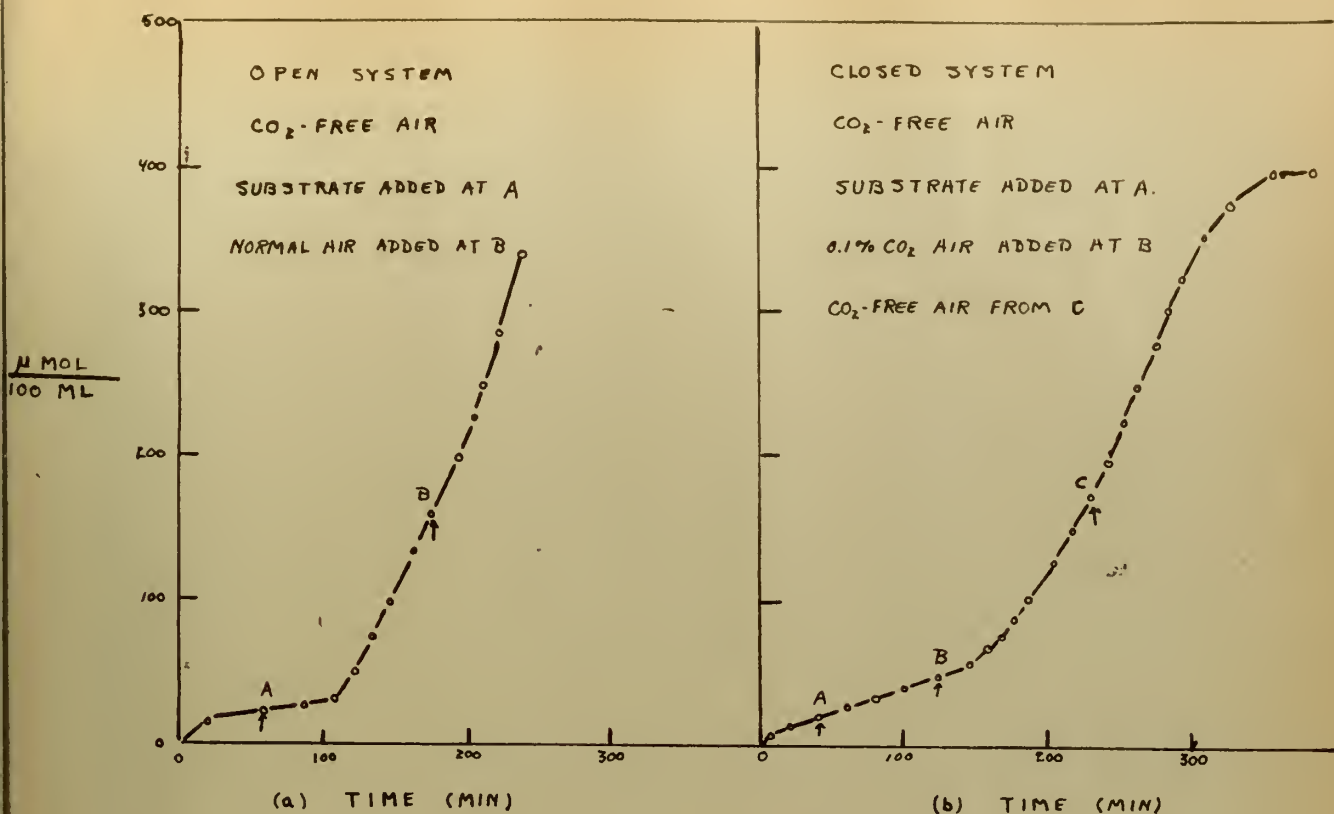


Figure 7. Examples of Propionate Metabolism

after initial activation. This could logically be explained by the CO_2 production from oxidations in the Krebs cycle, which could maintain the reactions once started.

Although several runs were not carried through to completion, it can be seen from those that were, that alkali production was not in all cases equivalent to the total amount of substrate furnished. This was similar to the results obtained in the two experiments using sodium pyruvate in the closed system. As before, no explanation can be offered for this behavior.

Conclusions which were reached during the investigations on propionate metabolism by *Rh. rubrum* were: (1) CO_2 is necessary for oxidative assimilation of sodium propionate in the dark, although in very small quantities;

- (2) Metabolic processes involving CO_2 are self-sustaining, once started;
- (3) Probable method of metabolism is by direct carboxylation of propionate to succinate, with sustaining action due to oxidations producing CO_2 within the Krebs cycle.

BIBLIOGRAPHY

1. Clayton, R. K. STUDIES IN THE PHOTOTAXIS OF RH. RUBRUM
Archiv fur Mikrobiologie, Bd 19, S 107-165
(1953)

2. Clayton, R. K. and PURPLE BACTERIA
Delbruck, M. Scientific American, 185, 5, Nov (1951)

3. Eisenberg, M. THE TRI-CARBOXYLIC ACID CYCLE IN RH. RUBRUM
J. Biol. Chem. 203, 815-36, (1953)

4. Friedeman, T. E. and THE DETERMINATION OF KETO ACIDS IN BLOOD AND
Haugen, G. E. URINE--J. Biol. Chem. 147, 415-42, (1943)

5. Glover, J., STUDIES ON THE METABOLISM OF PHOTOSYNTHETIC
Kamen, M. D. and BACTERIA. XII COMPARATIVE LIGHT AND DARK
Van Genderen, H. METABOLISM OF ACETATE AND CARBONATE BY RH.
RUBRUM--Archives of Biochem. and Biophysics,
35-36, 384-408, (1952)

6. Manten, A. PHOTOTAXIS, PHOTOTROPISM, AND PHOTOSYNTHESIS
IN PURPLE BACTERIA AND BLUE-GREEN ALGAE
Schotanus & Jens, Utrecht (1948)

7. Molisch, H. DIE PURPURBAKTERIEN NACH NEUEN UNTERSUCHUNGEN
G. Fisher, Jena, (1907)

8. Petrie, B. R., Jr. DEPENDENCE OF METABOLIC RATE ON OXYGEN CON-
CENTRATION IN RH. RUBRUM--Thesis, USNPS (1954)

9. van Niel, C. B. THE CULTURE, GENERAL PHYSIOLOGY, MORPHOLOGY,
AND CLASSIFICATION OF THE NON-SULFUR PURPLE
AND BROWN BACTERIA--Bact. Rev. 8, No. 1 (1944)

10. van Niel, C. B. in PHOTOSYNTHESIS IN PLANTS 437-95 (Frank, J.
and Loomis, W. F.) Iowa State College Free
Press (1949)

APPENDIX I

CULTURE AND PREPARATION OF THE BACTERIA

Rh. rubrum (strain S-1, VAN NIEL) was grown anaerobically (in 125 ml. cotton-stoppered florence flasks) in a light cabinet at 30-35°C. A liquid medium of 1.1% yeast extract, 0.05% MgCl_2 , 0.02% CaCl_2 , 0.1% $(\text{NH}_4)_2\text{SO}_4$, 0.07% $(\text{Na}_2\text{H}/\text{KH}_2)\text{PO}_4$ -(pH 7), 10% tap water and 88.7% distilled water was used for this cultivation. When the cultures were approximately four days old they were centrifuged and the bacteria resuspended in the following medium: 0.01% CaCl_2 , 0.04% MgCl_2 , 0.18% $(\text{NH}_4)_2\text{SO}_4$, 0.02% NaHCO_3 , 0.03% $(\text{Na}_2\text{H}/\text{KH}_2)\text{PO}_4$ -(pH 7), 0.09% substrate, and the balance, distilled water. The substrate was either sodium pyruvate or sodium propionate, depending on the study under investigation.

After adaptation for about 24 hours, the bacteria were centrifuged and washed with 100 ml. of .003M NaHCO_3 + .0005M phosphate buffer (pH 7). The turbidity of this suspension was measured by using a photoelectric cell with a red filter as described by Petrie[8]. Following a second centrifuging, the bacteria were suspended in the proper volume of .0005M phosphate buffer to obtain the desired turbidity (usually 2.0 ml. of cells/100 ml. suspension). During the runs with sodium propionate, .001M buffering was used both for the washing and the final suspension in order to minimize changes in pH following the addition of CO_2 .

One hundred ml. of this final suspension was used for a run using the open system and 150 ml. for the closed system. Substrate was added in each case to give a concentration of .005M. This was added either at the beginning of a run or shortly thereafter.

APPENDIX II

PYRUVATE ASSAY

The pyruvate concentration in the bacterial suspension was determined by the method of Friedeman and Haugen [4], with a modification as suggested by M. Doudoroff (verbal communication). This method is based on the reaction of sodium pyruvate with 2,4-dinitrophenylhydrazine, yielding a red-brown coloration in a solution made strongly alkaline with NaOH. While the reaction is not specific for pyruvic acid, it is specific for keto acids as a group. This was sufficient for purposes of this investigation due to the absence of other keto acids in a concentration that would interfere. The modification greatly simplified the procedure as it eliminated the extractions with organic solvent and Na_2CO_3 solution. The absence of interfering substances made this modification possible. The reagent used was 1/5 saturated 2,4-dinitrophenylhydrazine in 2N HCl. A saturated solution was first prepared by grinding 100 mg. of 2,4-dinitrophenylhydrazine in a mortar with increasing amounts of 2N HCl until 100 cc. of the acid had been added. The resultant suspension was filtered, diluted to 500 cc. with 2N HCl, and stored in a cold place until needed.

One tenth ml. of the bacterial suspension was added to one ml. of hydrazine reagent and 0.9 ml. distilled water in a regular size Klett test tube. After thorough mixing, the mixture was allowed to stand for five minutes. Then three ml. of distilled water and one ml. of 3N NaOH were added and thoroughly mixed with the suspension. The resulting solution stood for 30 minutes to permit full development of the characteristic color. The color was measured against a blank prepared by treating 0.1 ml. of the pyruvate-free suspension as above. In this experiment a Klett-Summerson

photoelectric colorimeter with a green filter was used for the determination. The concentration of pyruvate was determined by comparison with a standard prepared by adding 0.1 ml. of 0.005N sodium pyruvate and 0.1 ml. of pyruvate-free suspension to one ml. of the hydrazine reagent and 0.8 ml. distilled water and then treating as above.

Because of slow fading of both blank and sample, each determination was accompanied by a blank prepared simultaneously. Two cc. of the bacterial suspension was withdrawn before the substrate was added. A blank was drawn from this to accompany each determination made throughout the run.

The Klett-Summerson photoelectric colorimeter is so designed that the scale reading is directly proportional to the concentration of the substance being determined. The scale reading is a measure of the optical density of the colored solution as determined by a photoelectric cell. As the optical density is theoretically proportional to the concentration of colored substance¹, the scale readings are also proportional to the concentration. The relationship is linear in the range of 0-500 scale units. Above this maximum, the concentration of the colored substance is such that the optical density is no longer directly proportional to the concentration. The standard used gave a reading of approximately 500 units, while the samples during the runs gave lower readings. This is within the range of linearity for the method.

As the standard used was equivalent to 500 μ mol of sodium pyruvate in the original suspension, the amount of substrate in any sample could be calculated as:

$$\mu\text{mol pyruvate} = \frac{\text{reading} \times 500}{\text{reading}} \begin{matrix} \text{(sample)} \\ \text{(standard)} \end{matrix}$$

1. Beer's law.



Thesis

28489

E35 Ellingson

Development of a technique
for metabolic investigations
and its application to
studies with rhodospirillum
rubrum.

T
E

RETURN TO ACQUISITIONS DEPT.

Thesis

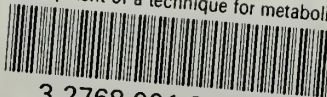
28489

E35 Ellingson

Development of a technique for
metabolic investigations and its
application to studies with
rhodospirillum rubrum.

thesE35

Development of a technique for metabolic



3 2768 001 89292 0

DUDLEY KNOX LIBRARY